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13. ABSTRACT (Maximum 200 words)		

We use olfactory receptor neurons and membrane fragments from the rat olfactory epithelium incorporated into planar bimolecular lipid membranes and patch-bilayers to define the initial electrochemical membrane events in chemoreception by the mammalian olfactory system. We have found and characterized a chemosensitive K<sup>+</sup> channel from rat olfactory epithelium homogenates which can be functionally reconstituted into artificial planar lipid bimolecular membranes. We have demonstrated that cAMP mimicked the effect of odorant. The statistical analysis of our patch-clamp data suggested the multiple mode of cAMP action on the single channel activity: (a) directly, and (b) via protein kinase system. We demonstrated, that chemosensitivity of functionally reconstituted olfactory receptor is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants in the medium bathing the membrane under control of the activity of cyclic nucleotide-processing enzymes.

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FUNCTIONAL RECONSTITUTION OF OLFACTORY RECEPTORS

FINAL REPORT

JUNE 17, 1994

U.S. ARMY RESEARCH OFFICE

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## A. STATEMENT OF THE PROBLEM STUDIED

We have proposed to define the initial electrochemical membrane events in chemoreception by the mammalian olfactory epithelium by study of olfactory receptor neurons and membrane fragments from rat olfactory epithelial homogenates incorporated into planar bimolecular lipid membranes and patch-bilayers.

The main objectives of this project were to define and describe the molecular mechanisms of the ion transport, associated with the initial events of olfaction; to define and characterize the electrochemical properties of the olfactory neurons, olfactory receptors reconstituted in bilayers, and ion mechanisms associated with primary events in olfactory reception; and to determine the regulatory mechanisms involved in function of olfactory receptor using biophysical and biochemical techniques.

## B. SUMMARY OF THE MOST IMPORTANT RESULTS

1. A simple technique for the construction of stable, essentially solvent-free bimolecular lipid membranes (BLM) of large surface area, using successive transfer of monolayers onto the aperture of a hydrostatically closed Teflon chamber has been developed (Vodyanoy & Murphy, 1982a; Vodyanoy et al., 1982, 1985; Vodyanoy, 1988, 1989b). The electrical capacitance of our membranes was  $0.8 \pm 0.1 \text{ uF/cm}^2$ ; the dc resistivity of the membrane (Vodyanoy et al., 1972, 1973) at  $25^\circ\text{C}$  was in the range of  $0.1\text{-}1 \text{ Gohm/cm}^2$ . A major advantage of the present method arises from the hydrostatically stabilized closed chamber upon which the membrane is formed. This results in an increase in the stability of the membrane to mechanical disturbances. This arises from the fact that a pressure drop in the open compartment is equilibrated by the pressure in the closed compartment, as water may be considered essentially incompressible at those low hydrostatic pressures. A patch-clamp technique ("tip dipping method") used for single channel measurements of the functionally reconstituted olfactory receptors (Vodyanoy, 1989a).

2. The steady-state conductance of BLM modified with rat olfactory epithelial homogenate became sensitive to very low concentrations in the nanomolar region of the odorant diethyl sulfide in the presence of adenosine triphosphate (ATP) and guanosine triphosphate (GTP) (Vodyanoy & Murphy, 1982b, 1983). The chemosensitivity was not observed when ATP and GTP were absent (Vodyanoy & Vodyanoy, 1987, 1987a; Vodyanoy, 1989b). The chemosensitive response was measured in terms of current flow at constant voltage. We observed a linear correlation ( $r = .84$ , slope = 2.2,  $N = 38$ ) between the stationary level of the membrane conductance evoked by the diethyl sulfide and the logarithm of its concentration. Other molecules which are less odorous, such as (+) and (-) carvone, also appear to activate a similar current flow across the membrane. Vesicles prepared from rat respiratory epithelium and added to the BLM in the same way as the olfactory vesicles showed no influence of diethyl sulfide up to millimolar concentrations. Membranes untreated with either vesicles also showed no detectable change in d-c conductance at millimolar diethyl sulfide concentration. We have found further that adenosine 3',5'-monophosphate (cAMP) mimicked the effects of the odorants, and the effect of cAMP was also dose dependent (Vodyanoy & Vodyanoy, 1987a). However, the reaction to the cAMP was observed when ATP and GTP were absent.

3. We found the existence of the single-channel fluctuations induced by odorants in large bimolecular lipid membranes treated with membrane homogenates of rat olfactory epithelium. In contrast to the fast channels registered by Labarca et. al., 1988, 1988a, Vodyanoy and Murphy, 1982b, 1983; Vodyanoy & Vodyanoy 1986 observed very slow channels. Addition of odorants such as diethyl sulfide in nanomolar concentrations increased the mean time in the open state from  $29.3 \pm 7.8 \text{ s}$  for spontaneously active channels to  $42.3 \pm 10.0 \text{ s}$  with the mean conductance about 60 pS. The odorant-activated channels were blocked by 4-aminopyridine, suggesting changes in  $\text{K}^+$  conductance.

4. When olfactory receptors were reconstituted in the small bilayers by the "tip dipping" method in asymmetric saline conditions ("inside out" configuration), addition of cAMP (without

ATP or GTP) to the pseudointracellular solution activated an ion channel with a conductance of about 70 pS (Vodyanoy & Vodyanoy, 1987b; Vodyanoy, 1989a, 1989b). The mean open time was about 1 sec. Subsequent addition of ATP did not change the unitary amplitude of this channel but caused a significant decrease of the mean open time to 6 msec. This activity was completely antagonized by protein kinase inhibitor. We analyzed statistically the temporal distribution of single ion channel fluctuations activated by cAMP and modulated by ATP (Vodyanoy & Vodyanoy, 1986). The histograms of the open state distribution showed a maximum. Study of the single-channel dwell time sequences revealed the existence of statistical dependency between adjacent open times. Further analysis of autocorrelation functions and Fourier spectra of the original and randomized open time sequences showed a positive correlation between open time events under presence of ATP. We suggest that cyclic gating scheme may result in correlation of successive dwell times, and the irreversible steps included in this cycle may require an energy supply to maintain the steady state (Vodyanoy & Vodyanoy, 1988; Vodyanoy, 1989a). We hypothesize that chemosensitivity of the functionally reconstituted olfactory receptor is manifested as a change in the mean open time of single channel events in response to small (subnanomolar) concentrations of the odorants. Furthermore this appears to be under control of cyclic nucleotide-processing enzymes.

5. We used bilayers treated with olfactory homogenates in "inside out" configuration to study cAMP-dependent conductance (Vodyanoy, 1989a, Vodyanoy, 1991). When cAMP added to "intracellular" solution the membrane conductance was increased. The subsequent addition of ATP into the same solution caused a small decrease of membrane conductance presumably due to an endogenous cAMP-dependent protein kinase. Forskolin added to pseudointracellular solution elicited an increase in membrane conductance similar to that produced by cAMP. This response required the presence of ATP and could be enhanced by theophylline. The response was not observed if ATP was replaced by ATP $\gamma$ S. The conductance increased by cAMP or Forskolin can be completely blocked by 50 mM BaCl $_2$ . In the presence of ATP addition of the catalytic subunit of the cAMP-activated protein kinase to the "cytosolic" side of the membrane inactivates the channels, presumably by phosphorylation. When ATP replaced by ATP $\gamma$ S the catalytic subunit was not effective. The porcine protein kinase inhibitor (inhibitor of cAMP-dependent protein kinase) elicited a dose and time-dependent increase of membrane conductance in a presence of ATP. The protein kinase (PK) added to pseudointracellular solution abolished the response to cAMP in a presence of ATP and it was not effective if ATP was replaced by ATP $\gamma$ S. So, our data indicate that cAMP-dependent protein kinase can be involved in modulation of ion channels reconstituted in bilayers from olfactory homogenates.

6. We demonstrated that chemosensitivity was manifest as a change in the mean open time of single channel events in response to the presence of odorants in the bathing media (Vodyanoy & Murphy, 1983). Our experiments support the hypothesis that the olfactory receptors mediate cation permeability via an increase in the intracellular concentration of cyclic AMP. Activation of the conductivity increase can be effected either directly by application of cyclic AMP or indirectly by a low dose of odorant in presence of ATP and GTP. We believe that phosphorylation modulates the mean open time in our reconstituted system. This suggests that cAMP regulates the channel activity in two ways: (a) directly, (b) *via* protein kinase system (Vodyanoy, 1989a). This dual control of the channel activity can explain the nature of very slow ion channels found in our early work (Vodyanoy & Murphy, 1983). As it was demonstrated later (Vodyanoy & Vodyanoy, 1987b; Vodyanoy, 1989a) cAMP could "open" the ion channel directly, or it could cause an activation of cAMP-dependent protein kinase that triggered a phosphorylation and "closure" of the channels. cAMP alone in our experiments activated a relatively slow (~1 sec) channel. When we facilitated a protein kinase activity an open time of this channel sharply decreased (~6 msec). On the contrary, the inhibition of the protein kinase caused a very slow or an indefinitely open channel (Vodyanoy, 1989a). We can speculate that our early observation of a very slow channel can be explained by the lack of adequate amounts

or the inhibition of some component necessary for channel phosphorylation. When we provide the necessary conditions we do observe a very fast ion channel activated by cAMP.

## C. LIST OF ALL PUBLICATIONS AND TECHNICAL REPORTS

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2. "Cyclic Nucleotide-gated Electrical Activity in Olfactory Receptors". V.Vodyanoy, in: Receptor and transduction mechanisms in taste and olfaction. Joseph G. Brand and John H. Teeter (Eds.). Marcel Dekker, New York, 1989, pp. 319-345.
3. "Surface properties of two rabbit lung lamellar body preparations with markedly different fatty acid profiles". V.Vodyanoy, G.L. Bluestone, and K.J. Longmuir. Biochim. Biophys. Acta, 1047, 284-289, 1990.
4. "Cyclic AMP-sensitive ion cannels in olfactory receptor cells." V. Vodyanoy. Chemical Senses, 16, 175-180, 1991
5. "Chiral recognition of odorant (+) and (-) carvone by phospholipid monolayers". Suram Pathirana, William C. Neely, Lawrence J. Myers, and Vitaly Vodyanoy. J. Am Chem. Soc., 114, 1404-1405, 1992.
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3. "Interaction of smail molecules with monolayers", Abstracts of the 1990 Meeting of the American Physical Society, 12-16 March 1990, Anaheim, California
4. "Surface properties of lung surfactant". V.Vodyanoy, G.L.Bluestone, and K.J.Longmuir. Proceedings of the 8th International Symposium on Surfactants in Solution. June 10-15, 1990, Gainesville, Florida
5. "Molecular recognition of optical isomers (+)- and (-)-carvone by phospholipid monolayers." Vitaly Vodyanoy, Suram Pathirana, Lawrence J. Myers, and William C. Neely. Biophys. J., 59, 636a, 1991.
6. "Valinomycin/stearic acid monolayers". S. Pathirana, K. Eckert, L. Giacomino, S. Ransone, L. Myers, W.C. Neely, and V. Vodyanoy, Abstracts of the 1992 Meeting of the Biophysical Society, 9-13 February 1992, Houston, Texas.
7. "Reconstitution of glutamate (AMPA) receptors in bilayers" Randy A. Hall, Michel Baudry, and Gary Lynch. A workshop-Symposium Sponsored by the US Army research Office, April 26-29, 1992, p.4

- 8 "Ligand-gated ion channel activity from rat brain AMPA receptors reconstituted in lipid bilayers." V. Vodyanoy, B.A. Bahr, V. Suppiramaniam, R.A. Hall, M. Hennergriff, K.Hoffman, M. Baudry, and G. Lynch. Abstracts of Fifth Conference on the Neurobiology of Learning and Memory. October 22-24, 1992. Irvine, California.
9. "Rat brain glutamate (AMPA) receptors reconstituted in lipid bilayers exhibit low and high conductance states." V. Vodyanoy, B.A. Bahr, V. Suppiramaniam, R.A. Hall, M. Kessler, M. Baudry, and G. Lynch. Abstracts. Society for Neuroscience. 22 Annual Meeting, October 25-30, 1992, Anaheim, California.

**D. LIST OF ALL PARTICIPATING SCIENTIFIC PERSONNEL SHOWING ANY  
ADVANCED DEGREE EARNED BY THEM WHILE EMPLOYED ON THE PROJECT.**

1. Vitaly Vodyanoy, Professor
2. Hiro Ito, MS, Physics
3. Suram Pathirana, PhD, Chemistry
4. Visnu Suppiramaniam, PhD, Biomedical Sciences

**E. REPORT OF INVENTIONS**

None

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